Water Air Soil Pollut (2020) 231: 252
https://doi.org/10.1007/s11270-020-04634-7

Exploring Elimination Kinetics of Four 5-Nitrofuran Derivatives by Microbes Present in Rural and Municipal Activated Sludge

Amanda Pacholak · Agata Zdarta · Robert Frankowski · Zefiryn Cybulski · Ewa Kaczorek

Received: 10 March 2020 / Accepted: 28 April 2020 / Published online: 15 May 2020

Abstract The wastewater treatment plants (WWTPs) are the biggest reservoirs of pharmaceutical residues discharged into the environment. Among many pharmaceuticals, derivatives of 5-nitrofuran, whose cytotoxicity and neurotoxicity have been proved, are widely used. The ability of such compounds to accumulate in water and sediments motivated us to analyze the ability of microbial communities of rural and municipal WWTPs to eliminate nitrofurantoin (NFT), nitrofurazone (NFZ), furaltadone (FTD), and furazolidone (FZD). Metagenomic analysis of microbial communities in rural and municipal activated sludge has provided information about the bacterial biodiversity in the WWTPs. In both samples, the most dominant phylum in terms of abundance was *Proteobacteria* followed by *Bacteroidetes*; however, microbial community of the municipal WWTP exhibited greater biodiversity than the one of the rural WWTP. The results of high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) analysis of the samples and elimination kinetic calculations allowed the determination of FZD, FTD, NFT, and NFZ elimination half-time varying from 104 to 327 h and test system first-order half-lives in the examined WWTP samples (from 31 to 231 h). Moreover, a comparison of the effectiveness of the microbials from two treatment plants, a rural one and a municipal one, revealed the poorer performance of the microbial communities from the smaller, rural WWTP in disposal of the analyzed pharmaceuticals, as after 24 days, the rural WWTP community was able to eliminate from 20 to 62% of 5-nitrofuran derivatives, while the municipal consortium removed over 85% of the compounds from the cultures.

Keywords Activated sludge · Metagenomic analysis · Nitrofurans · Nitrofurans elimination kinetics · Pharmaceuticals

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11270-020-04634-7) contains supplementary material, which is available to authorized users.

A. Pacholak · A. Zdarta · E. Kaczorek (✉)
Institute of Chemical Technology and Engineering, Poznan University of Technology, Berdychowo 4, 60-965 Poznan, Poland
e-mail: ewa.kaczorek@put.poznan.pl

R. Frankowski
Institute of Chemistry and Technical Electrochemistry, Poznan University of Technology, Berdychowo 4, 60-965 Poznan, Poland

Z. Cybulski
Department of Microbiology, The Greater Poland Cancer Centre, Garbary 15, 61-866 Poznan, Poland

1 Introduction

The presence of pharmaceutically active compounds (PhACs) in the natural environment has been documented in a number of reports and is recognized as one of the greatest environmental problems. A long-term, undesirable exposure of humans, aquatic and terrestrial organisms, and environmental microorganisms to PhACs may be dangerous and affect human health and ecological balance (Roberts and Bersuder 2006; Walters et al. 2010; Lacey et al. 2012; Kapelewska et al. 2018; Dong

et al. 2019; Zhao et al. 2019). One group of compounds that may be especially hazardous are 5-nitrofuran derivatives (5-NFs). Their most important representatives are nitrofurantoin (NFT), nitrofurazone (NFZ), furaltadone (FTD), and furazolidone (FZD) (Edhlund et al. 2006; Valera-Tarifa et al. 2013; Yu et al. 2013). The 5-NF derivatives make a group of chemotherapeutic compounds showing a wide-spectrum antibacterial and anti-protozoan activity. They were used in veterinary medicine as food additives to treat infected animals; however, because of their potential carcinogenic, mutagenic, and teratogenic properties, their use was prohibited in 1995 in the European Union countries. Nevertheless, they are still in use in many developing countries (Leston et al. 2011; Biošić et al. 2017; Bacanlı and Başaran 2019; Tolić et al. 2019). Moreover, selected 5-NFs, such as NFT or FZD, are still used to treat bacterial and protozoan infections in humans (Küng et al. 2019; Lewkowski et al. 2019; Ny et al. 2019). Importantly, these compounds show strong ability to accumulate in animal tissues and plants (Jeya Shakila et al. 2008; Leston et al. 2011; Hassan et al. 2013; Valera-Tarifa et al. 2013). They are also characterized by a relatively low tendency to adsorb onto sand and clay particles, which promotes their environmental mobility (Tolić et al. 2019). To date, a number of reports have been devoted to study the removal of PhACs at wastewater treatment plants (WWTPs) (Joss et al. 2005; Phillips et al. 2010; Phonsiri et al. 2019; Comber et al. 2019; Nguyen et al. 2019). Nevertheless, the activated sludge processes are often unable to complete the removal of pharmaceuticals (Musson and Townsend 2009). Activated sludge is a unique environment composed mainly of bacteria and protozoa. There are different strategies which may be employed in degradation studies. The first one involves the isolation of pure bacterial cultures with potential abilities to degrade xenobiotics. The other one includes DNA isolation and construction of a metagenomic library (Guo et al. 2017; Folch-Mallol et al. 2019). The latter is a novel, innovative approach which allows (i) better understanding of the role of selected microbes in ACS processes, (ii) isolation of new genes related to degradation, and (iii) design of degradation strategies, particularly in highly polluted environments (Guo et al. 2017; Folch-Mallol et al. 2019). Taking into account that each consortium is different and its composition depends on many factors (geographical location, season of the year, capacity of the WWTP, etc.), this new approach requires analysis of a number of different activated sludge consortia to describe similarities and differences between them and find effective solutions for their application in bioremediation processes. As literature lacks analyses of this type from central European countries, the main aim of this study was to characterize activated sludges collected from two WWTPs in Poland at molecular level using metagenomic analysis. Moreover, an important element of the research was analysis of degradation process of the most important 5-NF derivatives (nitrofurantoin, nitrofurazone, furazolidone, furaltadone) in terms of kinetic study and ability of microbes to remove these compounds.

2 Materials and Methods

2.1 Chemicals

Reagents of analytical grade, including NFT, NFZ, FZD, and FTD (Fig. 1), were purchased from Sigma-Aldrich (Poznan, Poland), and microbiological media were obtained from bioMérieux (Poland, Warsaw). All aqueous solutions were prepared using ultrapure water (Arium® pro, Sartorius, Kostrzyn, Poland).

2.2 Sample Collection and DNA Extraction

The samples were collected from two wastewater treatment plants (WWTPs) in Poland: municipal (M_WWTP) and rural (R_WWTP). Samples of 500 mL were taken aseptically from activated sludge bioreactors in March 2019. Detailed information about the WWTPs is summarized in Table 1.

The samples were transported to the laboratory within 1 h and aerated at room temperature for 24 h on a rotary shaker at 120 rpm (Chemland, Stargard, Poland). Afterward, the samples were sieved on 125 μm sieve to remove solid and large particles, centrifuged at 4500 *g* for 10 min, and then pellets were resuspended in mineral salt medium (composition [g L^{-1}]: $Na_2HPO_4 \cdot 2H_2O$ 7.0, KH_2PO_4 2.8, NaCl 0.5, NH_4Cl 1.0) and used for both DNA extraction and inoculation of microbial cultures in degradation tests. DNA was extracted according to the manufacturers' protocol (Sigma-Aldrich, GenElute® Bacterial DNA extraction kit). The quality of DNA was assessed using 1% agarose gel electrophoresis, and DNA concentrations were quantified by Multiskan Sky Microplate Spectrophotometer (Thermo Fisher

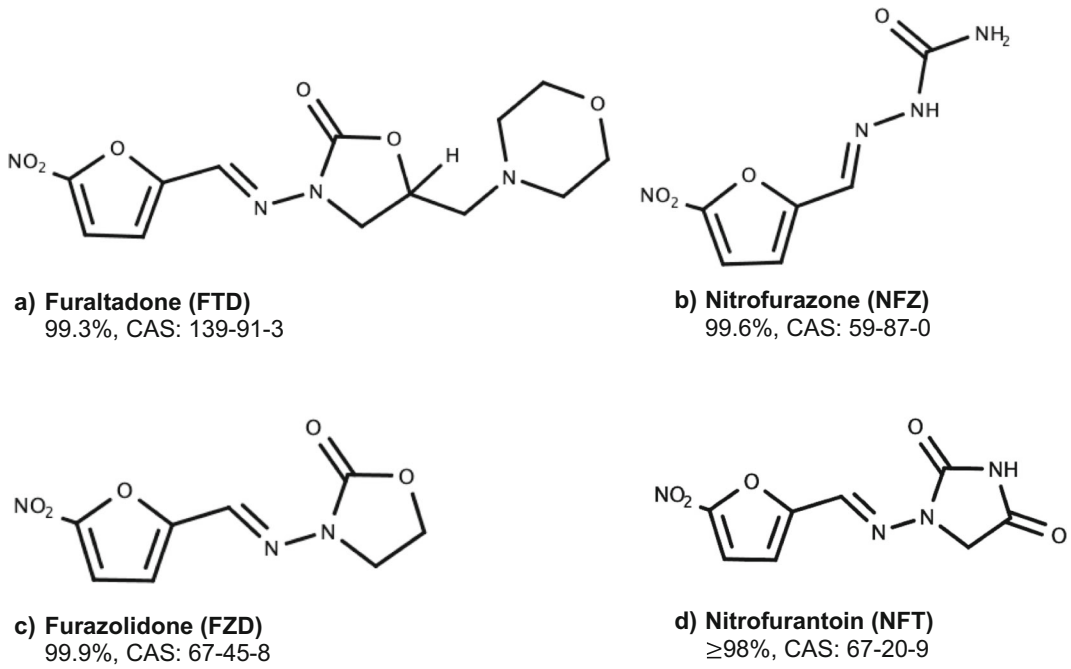

Fig. 1 Chemical structures of the analyzed 5-NF derivatives: **a** furaltadone (FTD), **b** nitrofurazone (NFZ), **c** furazolidone (FZD), **d** nitrofurantoin (NFT)

Scientific, Waltham, MA, USA). The DNA samples obtained were stored at 20 °C for further application.

2.3 16S rRNA Gene Sequencing and Microbial Communities' Analysis

16S rRNA metagenomic sequence library of V3-V4 regions of the DAN samples was prepared using 341 F and 785R primers and amplified with Q5 Hotstart High-Fidelity DNA Polymerase (NEBNext), according to manufacturers' protocol. After purification, the PCR products were sequenced using Illumina Hiseq 4000 by applying 2 × 250 nt pair-ended (PE) strategy using MiSeq Reagent Kit v2 (Illumina 2014). The data were analyzed using MiSeq Reporter (MSR) v2.6, protocol 16S metagenomics. Total reads for the samples ranged from 92,620 to 140,282. After quality filtering, the number of high-quality reads per sample ranged from 84,554 to 126,290. Taxonomy classification was performed on the basis of Greengenes v13_5, base, modified by Illumina, and the classification rate summary is presented in the supplementary material (Online Resource 1).

2.4 Removal of 5-NFs

Separate biological removal of four 5-NF representatives: nitrofurantoin (NFT), nitrofurazone (NFZ), furaltadone (FTD), and furazolidone (FZD) by R_WWTP and M_WWTP was tested. The liquid cultures were grown in 250-mL Schott Duran® laboratory glass bottles. They were established using 5 mL of inoculum (activated sludge sample), 0.1 mL of sodium succinate (20% aqueous

Table 1 Characteristics of WWTPs from which the samples were taken

Type of WWTP	Geographical location	WWTP operational area	WWTP max capacity	Additional information
R_WWTP	52°29′41.6″ N, 16°35′08.8″ E	Rural area with over 8000 citizens	1150 m^3/24h	Mechanical biological treatment plant
M_WWTP	52°25′53.1″ N, 16°57′31.8″ E	Urban area with a population of at least 500,000	50000 m^3/24h	Mechanical biological treatment plant with increased nutrient removal and full treatment of generated sewage sludge

R_WWTP, rural wastewater treatment plant; M_WWTP, municipal wastewater treatment plant

solution), and 0.1 mL of trace elements solution and 45 mL of the appropriate 5-NF derivative solution prepared in mineral salt medium. The initial concentration of the compounds tested was 5 mg L^{-1}. In parallel, the abiotic control samples were prepared. They contained a mineral salt medium instead of inoculum. All glassware and solutions (such as sodium succinate and medium) were autoclaved before using in the experiments.

In order to determine the content of the selected residual 5-NF in microbial cultures, small samples were collected at 6-h intervals. They were diluted in methanol and filtered using Captiva Syringe Filters (PTFE membrane, pore size 0.2 μm, diameter 13 mm, purchased from Agilent, Santa Clara, CA, USA) provided with an Injekt® Solo single-use syringe (B. Braun Melsungen AG, Hessen, Germany). Furthermore, they were analyzed qualitatively and quantitatively for the residual content of the selected compound using HPLC/MS-MS (UltiMate 3000 RSLC from Dionex, Sunnyvale, CA, USA). HPLC/MS-MS chromatograms, together with method's linearity parameters for each of the compound studied are available in the supplementary material (Online Resource 2).

Samples of 5 μL were injected onto a Gemini-NX C18 column (100 mm × 2.0 mm i.d.; 3 μm) from Phenomenex (Torrance, CA, USA) maintained at 35 °C. The flow rate of the mobile phase made of ammonium acetate ($5 \cdot 10^{-3}$ mol L^{-1}) in water and methanol was 0.3 mL min^{-1}. Gradient elution was performed by linearly increasing the methanol concentration expressed in percentage from 75 to 80% in 2 min and then linearly increasing the percentage to 100% in 1 min. The LC column effluent was directed to the API 4000 QTRAP triple quadrupole mass spectrometer (AB Sciex, Foster City, CA, USA) through the electrospray ionization source operating in the negative ion mode for NFT and the positive ion mode for the other compounds.

The dwell time for each mass transition detected in the MS/MS multiple reaction monitoring mode was 200 ms. The ions were detected using the following parameters: curtain gas, 10 psi; nebulizer gas, 40 psi; auxiliary gas, 40 psi; temperature, 400 °C; and collision gas, medium. The detected mass transitions and specific parameters of each analyte are presented in Table 2.

2.5 Kinetic Analysis, Mathematical Models, and Equations

The parameters describing the pharmaceuticals elimination kinetics were calculated using five kinetic modes. The number of cells was assumed as constant in time, as the substrate concentration in accordance with initial cell concentration was insufficient to produce significant increase in cell number (Birch et al. 2017). As the concentration of the substrate was lower than the saturation constant (K_S), the first-order and logistic models were tested, as well as Monod kinetics (no growth). Chemicals half-life was calculated on the basis of the pseudo-first-order equation (OECD 2002) (Eqs. (4) and (5)). Table 3 shows the models, equations, and rate constants used to characterize the elimination kinetics.

2.6 Statistical Analysis

The experiments were performed in three biological replicates. The results presented in the manuscript are the mean values calculated from three independent experiments, and the error bars represent the standard deviation calculated from three values. The calculations were performed using Statistica v13 (StatSoft, Cracow, Poland).

3 Results

3.1 Characterization of the Activated Sludges: Bacterial Biodiversity Evaluated by Metagenomic Analysis

The bacterial biodiversity of activated sludge samples collected from municipal (M_WWTP) and rural (R_WWTP) wastewater treatment plants was investigated by metagenomic analysis. Compositions of bacterial communities were determined at various taxonomic levels (Fig. 2), and the reads passing quality filtering exceeded 90% in all samples. As for the sludge sample R_WWTP, 95% of bacteria were classified at family level (120,100 reads), 86% at genus (108,522 reads), and 33% (41,741 reads) at species. When it comes to the sample M_WWTP, the values were 89%, 83%, and 37%, respectively, with the number of reads 75,053, 69,880, and 30,907. The filtered data show the dominance of the domain *Bacteria* in the samples (over 99%) followed by a small proportion of *Archaea* (around 0.01%).

The relative abundance of bacterial taxonomic groups found in both rural and municipal activated sludge is presented in Fig. 3 a and b. The charts show the taxonomic classifications at the level of phylum,

Table 2 Analytical background for 5-NF degradation

Analyte	ISV [V]	DP [V]	Multiple reaction monitoring transitions (precursor ion $[M-H]^-$ or $[M+H]^+$ m/z → product ion m/z)					
			Analytical	CE [eV]	CXP [V]	Confirmatory	CE [eV]	CXP [V]
NFT	− 4500	− 60	237 → 152	− 17	− 10	237 → 124	− 20	− 10
FZD	4500	60	226 → 122	29	6	226 → 95	21	4
NFZ	4500	60	199 → 182	17	11	199 → 156	19	9
FTD	4500	60	325 → 281	18	6	325 → 252	23	5

ISV, ion spray voltage; DP, declustering potential; Ce, collision energy; CXP, collision cell exit potential

class, and order which were identified above 1% abundance.

Altogether, 25 bacterial phyla were detected in R_WWTP. The most dominant phylum in the terms of abundance was *Proteobacteria* (69.3%), followed by *Bacteroidetes* (19.4%), *Actinobacteria* (3.8%), *Firmicutes* (3.5%), and *Verrucomicrobia* (1.3%). The other phyla were rare and accounted for less than 1.0%. The unclassified microbial community at the phylum level was found to have an average abundance of 1.2%. As for the other taxonomic categories, they include 48 class-level classifications, 97 order-level, 216 family-level, and 590 genus-level ones.

In *Proteobacteria* phylum, *Betaproteobacteria* (BP) represented the most abundant class (34.9%), followed by *Gammaproteobacteria* (GP, 20.1%) and *Alphaproteobacteria* (AP, 10.2%). Among BP, *Burkholderiales* and *Rhodocyclales* were the most common in the terms of the total abundance (26.3% and 8.5% of total bacteria present in the R_WWTP). The main order-level constituents of the other most abundant classes were *Pseudomonadales* (8.7%) and *Thiotrichales* (3.6%) in GP as well as *Rhodobacterales* (3.6%) in AP. *Bacteroidetes* constituting the second most abundant phylum were found to have the most dominant classes of *Sphingobacteriia* (13.8%) and *Flavobacteriia* (5.4%). When it comes to *Actinobacteria* phylum, the dominant class was that of *Actinobacteria* with *Actinomycetales* as the most common.

In M_WWTP (Fig. 3b), the number of bacterial phyla detected was 27. Similarly to R_WWTP, the most abundant one was *Proteobacteria* (40.0%) followed by *Bacteroidetes* (12.4%). However, the other most abundant phyla in M_WWTP were *Firmicutes* (12.4%), *Actinobacteria* (8.2%), *Tenericutes* (6.0%), *Spirochaetes* (5.9%), and *Chloroflexi* (2.6%). The other phyla were rare and accounted for less than 1.0%. The unclassified microbial community at the phylum level was found to have an average abundance of 4.8%. As for the other taxonomic categories, they included 55 class-level classifications, 107 order-level, 233 family-level, and 581 genus-level ones.

In *Proteobacteria* phylum, BP represented the most abundant class (14.9%), followed by GP (11.1%), AP (8.1%), and *Deltaproteobacteria* (DP, 4.7%). Among BP, *Burkholderiales* and *Rhodocyclales* were was the most common in terms of the total abundance (8.8% and

Table 3 Models, equations, and rate constants used in the calculations

Equation number	Model	–ds/dt=	Rate constants (units)
Eq. (1)	First-order	$k_1 S$	$k_1 = \mu_{max} X_0 / K_S$
Eq. (2)	Logistic	$k_3 S(S_0 + X_0\text{-}S)$	$k_3 = \mu_{max} / K_S$
Eq. (3)	Monod (no growth)	$k_0 S/(K_S + S)$	$k_0 = \mu_{max} X_0$
Eq. (4)	Pseudo-first order	$P = a(1\text{-}\exp[-k_1(t\text{-}c)])$	$k_1 = \mu_{max} X_0 / K_S$
Eq. (5)	Half-life time	$t_{1/2} = \ln 2/k_1$	–

k_1, first-order rate constant; S, concentration of the substrate; μmax, maximum specific growth rate; S_0, initial substrate concentration; X_0, initial bacterial density multiplied by a cell quota; K_S, saturation constant; k_3, logistic rate constant; k_0, Monod rate constant; P, concentration of product formed; a, extent of biodegradation (asymptote); t, time; c, lag time prior to the onset of biodegradation; and $t > c$; t1/2, half-life time of the analyzed compounds

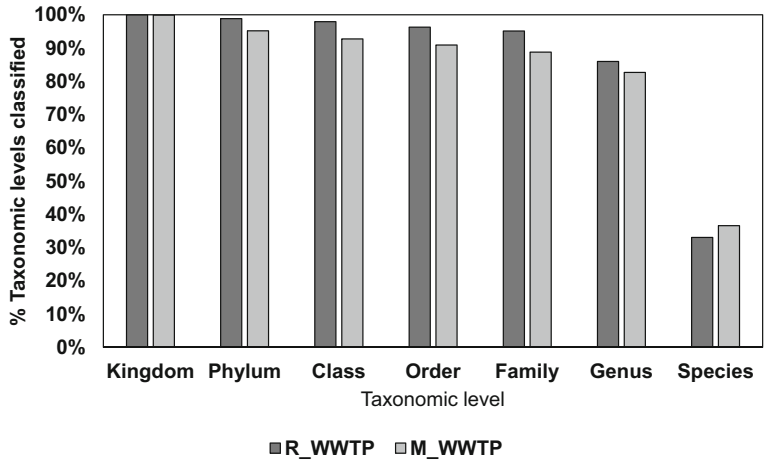

Fig. 2 Classification by taxonomic level in samples from rural (R_) and municipal (M_) wastewater treatment plants (WWTP)

5.5% of total bacteria present in the M_WWTP). The main order-level classifications found in the other phyla were as follows: GP, *Chromatiales* and *Enterobacteriales* (both 2.7%); AP, *Rhizobiales* (3.0%); and DP, *Myxococcales* (2.5%). *Bacteroidetes* phylum consisted of two main classes: *Sphingobacteria* with the order of *Sphingobacteriales* (12.9%) and *Flavobacteria* with *Flavobacteriales* (2.2%). When it comes to *Actinobacteria* phylum, the class of *Actinobacteria* with *Actinomycetales* was the most common (6.5%). However, *Lactobacillales* (3.6%) and *Bacillales* (3.4%) represented *Bacilli* as the main class of *Firmicutes*. The other most often identified classes and orders found in the M_WWTP activated sludge with their average abundances are as follows: *Clostridia* and *Clostridiales* (4.2%), *Mollicutes* and *Mycoplasmatales* (3.6%), *Acholeplasmatales* (2.9%), and *Leptospirae* and *Leptospirales* (4.0%).

3.2 Elimination of Nitrofuran Derivatives

An important element of the study was evaluation of the ability of the two activated sludges to remove four of the most important pharmaceuticals belonging to the nitrofuran group. The substrate decomposition by microorganisms present in the two WWTPs was evaluated using HPLC-MS/MS analysis. From each test system, nine samples were collected in the first 3 days of the tests in a few hour intervals, and the results illustrating the elimination of nitrofurans are presented in Fig. 4 a–d. Afterward, the results characterizing elimination of nitrofurans were used for the calculation of the parameters of kinetics of the processes by fitting to the five models of kinetics. The obtained parameters of nitrofurans elimination kinetics are summarized in Table 4.

As clearly follows from the figures consortia contained bacterial strains capable of using all nitrofurans (NFs) as a source of carbon and energy. However, the microorganisms present in municipal activated sludge were much more active in the elimination of these chemicals. In general, in the 72nd hour of the process, the highest removal efficiency was noted for furaltadone (FTD, 87.3%), while the process was carried out by the microorganisms from M_WWTP. The bacteria from R_WWTP were able to decompose 32.7% of the compound. As for the other pharmaceuticals, 63.0%, 61.3%, and 54.8% of FZD, NFT, and NFZ were removed in 72 h from the bacterial cultures containing the microorganisms from M_WWTP. Simultaneously, 22.9%, 23.3%, and 19.0% of the compounds in question were decomposed by the microorganisms from R_WWTP. What is worth mentioning, the greatest difference in removal efficiency between the bacteria coming from rural and municipal WWTP was noted for furaltadone (almost 55%), and for other pharmaceuticals, it was around 36–40%. Incomplete elimination of nitrofurans motivated us to perform additional sampling on the 24th day of cultivation in order to check whether long-term contact will enhance the removal efficiency (data not shown). An increase in elimination rate was noted in all samples except for the cultures containing NFZ and rural activated sludge. In the bacterial cultures with M_WWTP, almost complete removal of FTD and NFT was noticed; however, 85.6% and 89.8% elimination of NFZ and FZD was observed. As for R_WWTP, the final elimination rate on the 24th day of the process

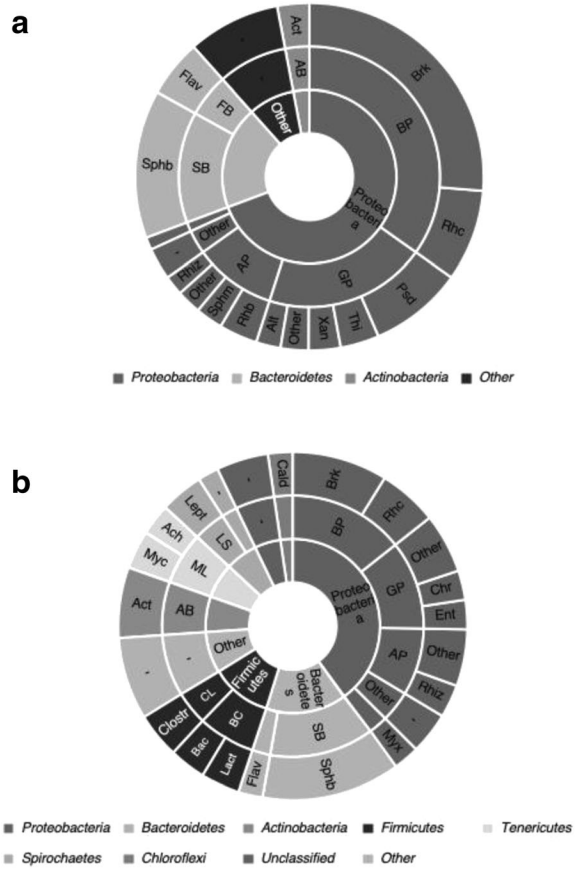

Fig. 3 The relative abundance of bacterial taxonomic groups in activated sludge: **a** rural wastewater treatment plant (R_WWTP), **b** municipal wastewater treatment plant (M_WWTP); BP, *Betaproteobacteria*; GP, *Gammaproteobacteria*; AP, *Alphaproteobacteria*; DP, *Deltaproteobacteria*; SB, *Sphingobacteria*; FB, *Flavobacteria*; AB, *Actinobacteria*; BC, *Bacilli*; CL, *Clostridia*; ML, *Mollicutes*; LS, *Leptospirae*; AN, *Anaerolineae*; Brk, *Burkholderiales*; Rhc, *Rhodocyclales*; Chr, *Chromatiales*; Ent, *Enterobacteriales*; Other, other; Rhiz, *Rhizobiales*; Myx, *Myxococcales*; Sphb, *Sphingobacteriales*; Flav, *Flavobacteriales*; Act, *Actinomycetales*; Lact, *Lactobacillales*; Bac, *Bacillales*; Clostr, *Clostridiales*; Myc, *Mycoplasmatales*; Ach, *Acholeplasmatales*; Lept, *Leptospirales*; Cald, *Caldilineales*; Psd, *Pseudomonadales*; Thi, *Thiotrichales*; Xan, *Xanthomonadales*; Alt, *Alteromonadales*; Rhb, *Rhodobacterales*; Sphm, *Sphingomonadales*

was 62.5% for NFT, 44.5% for FZD, and 49.0% for FTD. In general, the greatest increase in the removal efficiency was detected in the culture with NFT (almost 40% higher removal was observed).

On the basis of the LC-MS/MS results, the parameters of nitrofurans elimination kinetics of the analyzed substances were determined for both rural and municipal consortium-based systems. Table 4 presents the parameters calculated for the four nitrofuran derivatives, along with their half-live and elimination half-time in selected samples. The lag phase of the removal process varied between the samples but was more uniform for those based on the municipal activated sludge. In these samples, only nitrofurantoin had a lag phase equal to 84 h, and for the other drugs, it was 72 h. The results obtained for the systems with rural activated sludge can be divided into two groups: furazolidone and furaltadone with the lag phase of 96 h and nitrofurantoin and nitrofurazone with the lag phase close to 55 h. It can be also seen that the test systems' first-order rate constants are much lower for R_WWTP samples, which is reflected in the chemicals half-live and elimination half-time. The elimination half-time was the lowest for FTD in the system with M_WWTP and was equal to 104.5 h. Also, for this drug, the calculated first-order half-live was the lowest. For the other pharmaceuticals, the elimination half-time ranged between 137 and 149 h in the cultures with M_WWTP, and the first-order half-lives of the compounds studied in the test system were lower than 80 h. In comparison, the elimination half-times obtained for R_WWTP were, on average, twice higher and ranged from 269.3 to 327.0 h. The test systems with FZD and R_WWTP besides the longest elimination half-time were also characterized by the worst fit to the pseudo-first-order kinetic model. It is also worth mentioning that the rural activated sludge was also much less effective in the elimination of all tested chemicals.

4 Discussion

According to the results of calculations, 70–80% of nitrofuran derivatives used are released to the environment, and significant amounts of these drugs have been found in many environmental samples (Vass et al. 2008), making their removal an important issue. The results presented provide some information about bacterial biodiversity evaluated by metagenomic analysis of rural and municipal activated sludge and their ability to decompose the four most important nitrofuran-derived pharmaceuticals. Several reports have shown that the bacterial community composition in WWTP is strongly influenced by the WWTP geographical location, season, and influent wastewater (Zhang et al. 2012; Ibarbalz et al. 2016; Cydzik-Kwiatkowska and Zielińska 2016; Zhang et al. 2018b). However, most often than not, irrespective of WWTPs origin, many researchers have

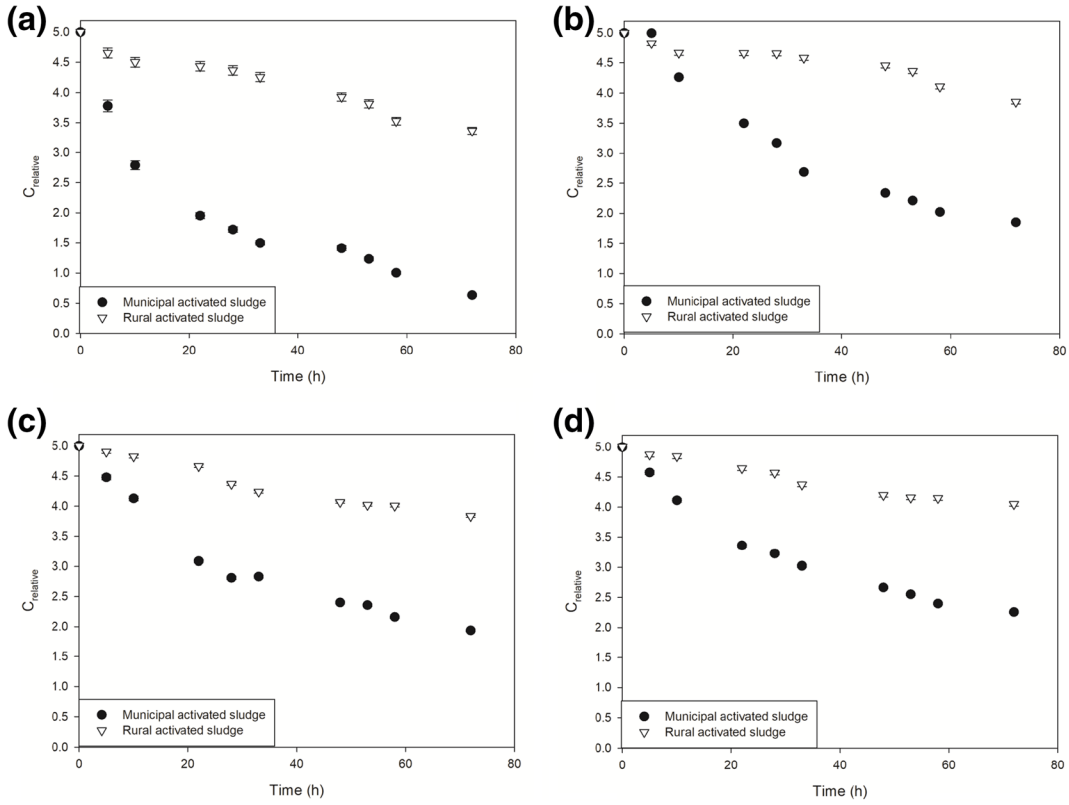

Fig. 4 Biological degradation of furaltadone (**a**), furazolidone (**b**), nitrofurantoin (**c**), and nitrofurazone (**d**). The graphs present the substrate removal efficiency from the bacterial cultures

noted the dominant role of *Proteobacteria*, followed by the members of *Firmicutes*, *Bacteroides*, and *Actinobacteria* in domestic WWTPs microbiomes (Ibarbalz et al. 2016; Zhang et al. 2017; Zhang et al. 2018a). The results of our studies also support these findings. The high abundance of the above-mentioned phyla may be explained by the fact that *Proteobacteria* are responsible for organic and nutrient removal. The other identified groups are involved in fermentation processes and come from with human fecal and sewage

Table 4 Degradation kinetics in activated sludge, lag phase, test system first-order rate constants, and degradation half-time

Chemical name	CAS number	Activated sludge source (M_WWTP/ R_WWTP)	Lag-phase (h)	Test system first-order rate constants	Fit	Test system first-order half-lives (*h*)	Degradation half-time (*h*)
FZD	67-45-8	M_WWTP	72	0.009	0.95	77.0	149.0
		R_WWTP	96	0.003	0.90	231.0	327.0
FTD	139-91-3	M_WWTP	72	0.022	0.95	31.5	104.5
		R_WWTP	96	0.004	0.95	173.3	269.3
NFT	67-20-9	M_WWTP	84	0.013	0.95	53.3	137.3
		R_WWTP	58	0.003	0.96	231.0	289.0
NFZ	59-87-0	M_WWTP	72	0.010	0.96	69.3	141.3
		R_WWTP	53	0.003	0.97	231.0	284.0

R_WWTP, rural wastewater treatment plant; M_WWTP, municipal wastewater treatment plant; FZD, furazolidone; FTD, furaltadone; NFT, nitrofurantoin; NFZ, nitrofurazone

samples (McLellan et al. 2010; Ye et al. 2012). The results of biological degradation indicate that more diverse consortia are more effective in toxic compounds removal (Thouand et al. 2011). Although the number of bacterial strains capable of specific xenobiotics decomposition is limited (Blok 2001), the more diverse inocula offer a greater chance to find the right one (Thouand et al. 2011). This finding is also supported by our results showing that the more diverse community of municipal WWTP is more effective in nitrofuran derivatives elimination. The importance of the daily capacity of WWTP and operational area must also be highlighted, as it also affects the density and variety of bacteria (Thouand et al. 2011; Zhang et al. 2012).

Different methods for nitrofurans derivatives removal have been reported in literature, including the physicochemical ones, such as photolysis (Edhlund et al. 2006), electrochemical degradation (Kong et al. 2015), or electron irradiation (Liu et al. 2007). The study of nitrofurantoin hydrolytic degradation at different pH and temperatures has shown the highest efficiency for NFT degraded in alkaline conditions at 60 °C (Biošić et al. 2017). The rate constants of degradation were higher at higher temperatures and in alkaline conditions, and the half-live of NFT was shortened to 0.4554 days in the most favorable conditions. Interestingly, when nitrofurantoin was hydrolyzed in neutral conditions and at temperature of 20 °C, its degradation half-live was as long as 300 days, but when the temperature was increased to 40°, the degradation half-live was shortened to around 11 days. This indicates that nitrofurantoin hydrolysis in natural environment is quite slow, and degradation by the activated sludge might be an important process to prevent nitrofuran derivatives release to the environment.

In the literature, there are a few reports on microbial degradation of different nitrofurans. Mohammad et al. (2018) noted 12–30% degradation of NFZ, FTD, and FZD by *Aspergillus species*. Zhang et al. (2013), on the other hand, described the degradation of FZD by pure bacterial strains such as *A. calcoaceticus* T32, *P. putida* SP1, and *P. mirabilis* V7. The strains used in their study were able to degrade from over 95% (*A. calcoaceticus* T32) to 82% (*P. mirabilis* V7) of FZD within 3 days (Zhang et al. 2013). Our findings seem to be consistent with these data as both wastewater activated sludge communities were able to decompose the analyzed nitrofurans. An interesting finding is a big difference in this process efficiency between the samples based on municipal and rural WWTPs sludges. Taking into account the differences in the community structure, revealed by metagenome analysis, the differences in biodiversity in the sludge samples from rural and municipal WWTP can be the main factor affecting microbial ability to biodegrade the analyzed 5-NF derivatives. Zhang et al. (2018a) who analyzed degradation of textile dyes in three wastewater treatment plants also observed significant differences in selected dyes removal between the samples coming from different WWTPs. The authors suggest that increasing abundance of selected pollutants in WWTP influx enhances bacterial adaptation to them. This might be a possible explanation of higher rates of elimination of NFT, NFZ, FTD, and FZD in municipal WWTP, characterized by higher capacity and operational area.

Our previous kinetic studies performed for pure strains on nitrofurantoin fitted better to the Monod model, based on continuous growth of cells (Pacholak et al. 2019). However, activated sludge as a complex community has more variable properties and could not be characterized with simple growth rate measurements. For this reason, it was assumed that nitrofurans elimination was proportional to the disappearance of the substrate, and the results showed a stronger correlation to the pseudo-first-order model than to the other tested models.

5 Conclusion

The study has provided information about the bacterial biodiversity in the rural and municipal activated sludge and their ability to decompose the four most important nitrofuran-derived pharmaceuticals. The most dominant phyla in both R_WWTP and M_WWTP were *Proteobacteria* and *Bacteroidetes*. The municipal activated sludge exhibited greater biodiversity and stronger ability to degrade nitrofurans than the rural activated sludge. The degradation kinetics model was best described by the first-order equation for all pharmaceuticals studied and enabled calculation of the tested chemicals half-live and elimination half-time.

Funding Information This work was supported by the National Science Centre, Poland (Grant no. 2017/27/B/NZ9/01603).

References

Bacanlı, M., & Başaran, N. (2019). Importance of antibiotic residues in animal food. *Food and Chemical Toxicology, 125*, 462–466. https://doi.org/10.1016/j.fct.2019.01.033.

Biošić, M., Škorić, I., Beganović, J., & Babić, S. (2017). Nitrofurantoin hydrolytic degradation in the environment. *Chemosphere, 186*, 660–668. https://doi.org/10.1016/j.chemosphere.2017.08.011.

Birch, H., Andersen, H. R., Comber, M., & Mayer, P. (2017). Biodegradation testing of chemicals with high Henry's constants – Separating mass and effective concentration reveals higher rate constants. *Chemosphere, 174*, 716–721. https://doi.org/10.1016/j.chemosphere.2017.02.003.

Blok J. (2001). A quest for the right order: Biodegradation rates in the scope of environmental risk assessment of chemicals. PhD Thesis University Utrecht. ISBN 90–74718–07-8, pp. 174.

Comber, S., Gardner, M., Sörme, P., & Ellor, B. (2019). The removal of pharmaceuticals during wastewater treatment: Can it be predicted accurately? *Science of the Total Environment, 676*, 222–230. https://doi.org/10.1016/j.scitotenv.2019.04.113.

Cydzik-Kwiatkowska, A., & Zielińska, M. (2016). Bacterial communities in full-scale wastewater treatment systems. *World Journal of Microbiology and Biotechnology, 32*, 66. https://doi.org/10.1007/s11274-016-2012-9.

Dong, P., Wang, H., Fang, T., Wang, Y., & Ye, Q. (2019). Assessment of extracellular antibiotic resistance genes (eARGs) in typical environmental samples and the transforming ability of eARG. *Environment International, 125*, 90–96. https://doi.org/10.1016/j.envint.2019.01.050.

Edhlund, B. L., Arnold, W. A., & McNeill, K. (2006). Aquatic photochemistry of nitrofuran antibiotics. *Enviromental Science & Technology, 40*, 5422–5427. https://doi.org/10.1021/es0606778.

Folch-Mallol, J. L., Zárate, A., Sánchez-Reyes, A., & López- Lara I.M. (2019). Expression, purification, and characterization of a metagenomic thioesterase from activated sludge involved in the degradation of acylCoA-derivatives. *Protein Expression Purification, 159*, 49–52. https://doi.org/10.1016/j.pep.2019.03.008.

Guo, J., Ni, B.-J., Han, X., Chen, X., Bond, P., Peng, Y., & Yuan, Z. (2017). Unraveling microbial structure and diversity of activated sludge in a full-scale simultaneous nitrogen and phosphorus removal plant using metagenomic sequencing. *Enzyme and Microbial Technology, 102*, 16–25. https://doi.org/10.1016/j.enzmictec.2017.03.009.

Hassan, M. N., Rahman, M., Hossain, M. B., Hossain, M. M., Mendes, R., & Nowsad, A. A. K. M. (2013). Monitoring the presence of chloramphenicol and nitrofuran metabolites in cultured prawn, shrimp and feed in the southwest coastal region of Bangladesh. *Egyptian Journal of Aquatic Research, 39*, 51–58. https://doi.org/10.1016/j.ejar.2013.04.004.

Ibarbalz, F. M., Orellana, E., Figuerola, E. L. M., & Erijman, L. (2016). Shotgun metagenomic profiles have a high capacity to discriminate samples of activated sludge according to wastewater type. *Applied of Environmental Microbiology, 82*, 5186–5196. https://doi.org/10.1128/AEM.00916-16.

Illumina (2014). MiSeqReporte metagenomics workflow reference guide part # 15042317 Rev. D.

Jeya Shakila, R., Saravanakumar, R., Shanmugapriya, E., & Jeyasekaran, G. (2008). Detection of furazolidone residues by microbial assay in thermally processed and cold stored shrimp. *Journal of Aquatic Food Product Technology, 17*, 156–172. https://doi.org/10.1080/10498850801937141.

Joss, A., Keller, E., Alder, A. C., Göbel, A., McArdell, C. S., Ternes, T., & Siegrist, H. (2005). Removal of pharmaceuticals and fragrances in biological wastewater treatment. *Water Research, 39*, 3139–3152. https://doi.org/10.1016/j.watres.2005.05.031.

Kapelewska, J., Kotowska, U., Karpińska, J., Kowalczuk, D., Arciszewska, A., & Świrydo, A. (2018). Occurrence, removal, mass loading and environmental risk assessment of emerging organic contaminants in leachates, groundwaters and wastewaters. *Microchemical Journal, 137*, 292–301. https://doi.org/10.1016/j.microc.2017.11.008.

Kong, D., Liang, B., Yun, H., Ma, J., Li, Z., Wang, A., & Ren, N. (2015). Electrochemical degradation of nitrofurans furazolidone by cathode: Characterization, pathway and antibacterial activity analysis. *Chemical Engineering Journal, 262*, 1244–1251. https://doi.org/10.1016/J.CEJ.2014.10.094.

Küng, E., Fürnkranz, U., & Walochnik, J. (2019). Chemotherapeutic options for the treatment of human trichomoniasis. *International Journal of Antimicrobial Agents, 53*, 116–127. https://doi.org/10.1016/j.ijantimicag.2018.10.016.

Lacey, C., Basha, S., Morrissey, A., & Tobin, J. M. (2012). Occurrence of pharmaceutical compounds in wastewater process streams in Dublin, Ireland. *Environmental Monitoring Assess, 184*, 1049–1062. https://doi.org/10.1007/s10661-011-2020-z.

Leston, S., Nunes, M., Viegas, I., Lemos, M. F. L., Freitas, A., Barbosa, J., Ramos, F., & Pardal, M. A. (2011). The effects of the nitrofuran furaltadone on *Ulva lactuca*. *Chemosphere, 82*, 1010–1016. https://doi.org/10.1016/j.chemosphere.2010.10.067.

Lewkowski, J., Rogacz, D., & Rychter, P. (2019). Hazardous ecotoxicological impact of two commonly used nitrofuran-derived antibacterial drugs: Furazolidone and nitrofurantoin. *Chemosphere, 222*, 381–390. https://doi.org/10.1016/j.chemosphere.2019.01.144.

Liu, Z., Zhang, H., & Liu, Y. (2007). Effect of electron irradiation on nitrofurans and their metabolites. *Radiation Physics and Chemistry, 76*, 1903–1905. https://doi.org/10.1016/J.RADPHYSCHEM.2007.02.084.

McLellan, S. L., Huse, S. M., Mueller-Spitz, S. R., Andreishcheva, E. N., & Sogin, M. L. (2010). Diversity and population structure of sewage-derived microorganisms in wastewater treatment plant influent. *Environmental Microbiology, 12*, 378–392. https://doi.org/10.1111/j.1462-2920.2009.02075.x.

Mohammad, N. S., Safian, M. F., Ariffin, S. H. Z., & Ariffin, Z. Z. (2018). Biotransformation of nitrofurans antibiotics by *Aspergillus* species - residual antibacterial activity. *Malaysian Journal of Biochemistry & Molecular Biology, 2*, 28–33.

Musson, S. E., & Townsend, T. G. (2009). Pharmaceutical compound content of municipal solid waste. *Journal of Hazardous Materials, 16*, 730–735. https://doi.org/10.1016/j.jhazmat.2008.05.089.

Nguyen, P. M., Afzal, M., Ullah, I., Shahid, N., Baqar, M., & Arslan, M. (2019). Removal of pharmaceuticals and personal care products using constructed wetlands: Effective plant-bacteria synergism may enhance degradation efficiency. *Environmental Science and Pollution Research, 26*, 21109–21126. https://doi.org/10.1007/s11356-019-05320-w.

Ny, S., Edquist, P., Dumpis, U., Gröndahl-Yli-Hannuksela, K., Hermes, J., Kling, A.-M., Klingeberg, A., Kozlov, R., Källman, O., Lis, D. O., Pomorska-Wesołowska, M., Saule, M., Wisell, K. T., Vuopio, J., Palagin, I., & NoDARS UTIStudy Group. (2019). Antimicrobial resistance of *Escherichia coli* isolates from outpatient urinary tract infections in women in six European countries including Russia. *Journal of Global Antimicrobial Resistance, 17*, 25–34. https://doi.org/10.1016/j.jgar.2018.11.004.

OECD. (2002). *Detailed review paper on biodegradability testing*. OECD Series on Testing and Assessment, No. 2, OECD Publishing, Paris. https://doi.org/10.1787/9789264078529-en.

Pacholak, A., Smułek, W., Zgoła-Grześkowiak, A., Kaczorek, E., Pacholak, A., Smułek, W., Zgoła-Grześkowiak, A., & Kaczorek, E. (2019). Nitrofurantoin—Microbial degradation and interactions with environmental bacterial strains. *International Journal of Environmental Research and Public Health, 16*, 1526. https://doi.org/10.3390/ijerph16091526.

Phillips, P. J., Smith, S. G., Kolpin, D. W., Zaugg, S. D., Buxton, H. T., Furlong, E. T., Esposito, K., & Stinson, B. (2010). Pharmaceutical formulation facilities as sources of opioids and other pharmaceuticals to wastewater treatment plant effluents. *Environmental Science & Technology, 44*, 4910–4916. https://doi.org/10.1021/es100356f.

Phonsiri, V., Choi, S., Nguyen, C., Tsai, Y.-L., Coss, R., & Kurwadkar, S. (2019). Monitoring occurrence and removal of selected pharmaceuticals in two different wastewater treatment plants. *SN Applied Sciences, 1*, 798. https://doi.org/10.1007/s42452-019-0774-z.

Roberts, P. H., & Bersuder, P. (2006). Analysis of OSPAR priority pharmaceuticals using high-performance liquid chromatography-electrospray ionisation tandem mass spectrometry. *Journal of Chromatography A, 1134*, 143–150. https://doi.org/10.1016/j.chroma.2006.08.093.

Thouand, G., Durand, M. J., Maul, A., Gancet, C., & Blok, H. (2011). New concepts in the evaluation of biodegradation/persistence of chemical substances using a microbial inoculum. *Frontiers in Microbiology, 2*, 164. https://doi.org/10.3389/fmicb.2011.00164.

Tolić, K., Mutavdžić, P. D., Židanić, D., & Runje, M. (2019). Nitrofurantoin in sediments and soils: Sorption, isotherms and kinetics. *Science of the Total Environment, 681*, 9–17. https://doi.org/10.1016/j.scitotenv.2019.05.054.

Valera-Tarifa, N. M., Plaza-Bolaños, P., Romero-González, R., Martínez-Vidal, J. L., & Garrido-Frenich, A. (2013). Determination of nitrofuran metabolites in seafood by ultra-high performance liquid chromatography coupled to triple quadrupole tandem mass spectrometry. *Journal of Food Composition and Analysis, 30*, 86–93. https://doi.org/10.1016/j.jfca.2013.01.010.

Vass, M., Hruska, K., & Franek, M. (2008). Nitrofuran antibiotics: A review on the application, prohibition and residual analysis. *Veterinary Medicine, 53*, 469–500.

Walters, E., McClellan, K., & Halden, R. U. (2010). Occurrence and loss over three years of 72 pharmaceuticals and personal care products from biosolids-soil mixtures in outdoor mesocosms. *Water Research, 44*, 6011–6020. https://doi.org/10.1016/j.watres.2010.07.051.

Ye, L., Zhang, T., Wang, T., & Fang, Z. (2012). Microbial structures, functions, and metabolic pathways in wastewater treatment bioreactors revealed using high-throughput sequencing. *Environmental Science & Technology, 46*, 13244–13252. https://doi.org/10.1021/es303454k.

Yu, W. H., Chin, T. S., & Lai, H. T. (2013). Detection of nitrofurans and their metabolites in pond water and sediments by liquid chromatography (LC)-photodiode array detection and LC-ion spray tandem mass spectrometry. *International Biodeterioration and Biodegradation, 85*, 517–526. https://doi.org/10.1016/j.ibiod.2013.03.015.

Zhang, B., Xu, X., & Zhu, L. (2017). Structure and function of the microbial consortia of activated sludge in typical municipal wastewater treatment plants in winter. *Scientific Reports, 7*, 17930. https://doi.org/10.1038/s41598-017-17743-x.

Zhang, B., Xu, X., & Zhu, L. (2018a). Activated sludge bacterial communities of typical wastewater treatment plants: Distinct genera identification and metabolic potential differential analysis. *AMB Express, 8*, 184. https://doi.org/10.1186/s13568-018-0714-0.

Zhang, B., Yu, Q., Yan, G., Zhu, H., Xu, X. Y., & Zhu, L. (2018b). Seasonal bacterial community succession in four typical wastewater treatment plants: Correlations between core microbes and process performance. *Scientific Reports, 8*, 4566. https://doi.org/10.1038/s41598-018-22683-1.

Zhang, T., Shao, M.-F., & Ye, L. (2012). 454 pyrosequencing reveals bacterial diversity of activated sludge from 14 sewage treatment plants. *The ISME Journal, 6*, 1137–1147. https://doi.org/10.1038/ismej.2011.188.

Zhang, W., Niu, Z., Yin, K., Liu, F., & Chen, L. (2013). Degradation of furazolidone by bacteria *Acinetobacter calcoaceticus* T32, *Pseudomonas putida* SP1 and *Proteus mirabilis* V7. *International Biodeterioration and Biodegradation, 77*, 45–50. https://doi.org/10.1016/j.ibiod.2012.11.006.

Zhao, Y., Cho, C.-W., Cui, L., Wei, W., Cai, J., Wu, G., Wu, G., & Yun, Y.-S. (2019). Adsorptive removal of endocrine-disrupting compounds and a pharmaceutical using activated charcoal from aqueous solution: Kinetics, equilibrium, and mechanism studies. *Environmental Science and Pollution Research, 26*, 33897–33905. https://doi.org/10.1007/s11356-018-2617-7.